Appendix A

Changes in the paragraph starting page 6, line 7:

Figure 5 shows the cleavage of purified extracellular matrix (ECM) proteins. Figure 5, A After five minutes of protease incubation with VN, degredation products could not be identified by Coomassie blue staining. Figure 5, B Incubation of FN with the protease for up to 12 hours did not result in formation of additional degredation products. Figure 5, C No significant cleavage of human laminin was observed under the experimental conditions assayed.

Changes in the paragraph starting page 6, line 9:

Figure 6 shows the induction of cytopathic effect and fibronectin cleavage in human umbilical vein endothelial cells (HUVEC) cultures. Figure 6, A Western immunoblot analysis of cells in the absence of protease, or treated with boiled protease for up to 8 hours, showed no detectable FN degredation. Figure 6, B By three hours after protease addition, zones of clearing occurred in the cell monolayer.

Changes in the paragraph starting on page 32, line 13, starting "To create a stable zymogen....," and ending on page 33, line 12:

To create a stable zymogen to facilitate crystallographic studies and generate enzymatically deficient or inactive protease for structure-function studies, mutant forms of the cysteine protease protein are made and characterized. A targeted mutagenesis scheme creates changes that: (i) disrupt protease activity; (ii) prevent zymogen processing; (iii) prevent substrate binding; and (iv) alter immunoreactivity. Amino acids are changed to structurally neutral alanine. A mutant protein that lacks protease activity, but which retains antigenicity, is generated by mutagenesis of the single cysteine residue (Cys-192->Ala-192)

at the catalytic site of the molecule. Also, His-340 and [Gin]Gln-185 and Asn-356 are mutagenized. These three changes are epistatic to the Cys-192 mutation, but may alone exhibit altered activity. Trp-357, thought to be involved in substrate binding and similarly positioned within papain, will also be targeted. A stable zymogen precursor is also created by mutating residues surrounding the protease cleavage site at Lys-145. In addition, mutagenesis of Cys-192 may prevent autoproteolysis, as occurred for a Cys->Ser mutant of papain, the prototype cysteine protease. Other mutagenesis targets include a putative nucleotide binding domain (SEQ ID NO:17) (GVGKVG) and a potential collagen docking region (SEQ ID NO:18) [$(GXX)_3$] within carboxy terminal portion of the protein. Site-directed mutagenesis is used, by the charged-to-alanine-scanning method, to substitute positively and negatively charged amino acids (often involved in recognition and activity) with alanine. Many of the charged residues (14 lysine, 7 arginine, 12 aspartate, and 7 glutamate residues in the mature peptide) are expected to lie on the surface of the cysteine protease structure, and some are expected to define epitopes on the molecule. In particular, a region of charged amino acids, from 307 to 321 (8/15 charged), is examined; this region includes the site of *speB2* and *speB4* amino acid substitutions. Residues in antigenic regions identified in the epitope mapping studies are also mutated.